

Calls of the Large Odorous Frog (*Odorrana graminea*) Show Nonlinear and Individual Characteristics

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Abstract Male large odorous frogs (*Odorrana graminea*) have been shown to have a rich vocal repertoire and their calls contain harmonics that extend into the ultrasonic range, much like calls of male *O. tormota*, a sympatric species. Whereas vocalizations of male *O. tormota* exhibit nonlinear characteristics and contain individual signatures, it is unclear whether or not those of *O. graminea* do. We carried out an extensive study of the vocalizations of male *O. graminea* with the goal of investigating whether they: (1) contained nonlinear phenomena (NLP), (2) carried individual signatures. We recorded and analyzed 1770 vocal calls from nine males. Their calls were classifiable into two distinct categories (one-note calls and multi-note calls). Of these, one-note complex/steep-frequency modulation call was the most abundant call-type. These calls showed diverse frequency modulation patterns, and abundant presence of NLP. Various basic spectral and temporal parameters of such calls were individually distinct. Kruskal-Wallis Analysis of Rank test showed that the calls from individual males differed significantly. Subsequent discriminant function analysis (DFA) showed that 54.7% of the calls could be correctly assigned to individual. These results suggested that male *O. graminea* might be able to discriminate neighboring frogs from strangers acoustically. However, further research is necessary to demonstrate this capacity behaviorally.

Keywords Anurans, call-type, individual signatures, nonlinear phenomena, sound communication

1. Introduction

The Large Odorous frog (*Odorrana graminea*) is an arboreal species sympatric with *O. tormota* inhabiting noisy streams and waterfalls in select regions of China (Chen, 1991). Their calls are classifiable into six basic call-types and most of them contain harmonics that extend into the ultrasonic range (Shen *et al.*, 2011). The most common call-type is frequency modulation (FM) calls with overt frequency modulation throughout the duration of the call.

A notable difference between the vocalizations of *O. graminea* and *O. tormota* is the occurrence of nonlinear phenomena (NLP). NLP refers to four acoustic characteristics not found in vocalizations produced by linear sound production systems, i.e., frequency jump, subharmonic, deterministic chaos, or biphonation (Wilden *et al.*, 1998). Frequency jump refers to an abrupt or rapid change within a few milli seconds, in the call fundamental frequency (f_0); subharmonics refers to harmonic segments of a call where there is energy at 0.5, 0.33, 0.25, 0.2, or 0.166 f_0 ; deterministic chaos refers to segments of a call with noise-like spectra; biphonation refers to a bifurcation of the f_0 or of the overtones. Whereas 92% of long calls of male (Feng *et al.*, 2009b) and 39% of female *O. tormota* vocalizations (Zhang *et al.*, 2017) contain at least one NLP component, there is so far no evidence of NLP in the vocalizations of male *O. graminea* for any of the reported call-types (Shen *et al.*, 2011).

During our longitudinal study of the vocalizations of male and female *O. tormota* in Huangshan (Anhui, China) over the last eight years (Zhang *et al.* 2015, 2016, 2017, 2020), we have also collected a small sample of the vocalizations of male *O. graminea* as the two species show partial overlaps in their reproductive periods and calling sites. Analysis of these vocalizations revealed that some of their calls exhibit frequency jumps and/or subharmonics. Furthermore, our pilot field observations have also revealed that male *O. graminea* can

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maintain the same territorial for a single reproductive season (territorial's radius < 0.3 m) and territorial male commonly have three to five territorial neighbors. Meanwhile, several cases of male-male physical combat were also observed. Thus, male *O. graminea* appear to display "dear enemy" phenomenon (Fisher, 1954; Temeles, 1994), a phenomenon frequently observed in various groups of animals especially in territorial animals, e.g., birds, mammals, fish, amphibians, and insects (Temeles, 1994; Briefer *et al.*, 2008; Bee *et al.*, 2016). Such animals discriminate familiar neighbors from unknown strangers using a variety of sensory cues, and they respond more aggressively to strangers. By recognizing neighbors and respecting established territorial boundaries, individuals can conserve time and energy that would otherwise be expended in unnecessary territorial defense (Falls, 1982; Temeles, 1994). It is possible that male *O. graminea* can discriminate familiar neighbors from strangers through their vocalizations.

Results of these casual observations motivated us to carry out a thorough study to investigate whether the vocalizations of male *O. graminea* (1) contained NLP and (2) carried individual signatures which allowed them to discriminate neighbors from strangers acoustically.

2. Materials and Methods

2.1. Study site Field study was performed from 26 April 2015 to 28 May 2015, in the village Fuxi (118°8'44.89"E, 30°5'1.61"N; datum = WGS84; Elevation: 600 m), in Huangshan, China. The ambient temperature and humidity at the study site at evening hours ranged from 15°C to 20.5°C and from 72% to 100%, respectively. During the study period, male *O. graminea* emerged from their daytime hiding places to call nightly (from 1900 h to 2400 h) from large boulders in the middle of select sections of a local stream (Fu Creek). The calling activity was dependent on the ambient temperature and humidity; chorus weakened substantially or vanished when the temperature dropped below 15°C, or when the humidity was < 70%. Their calling sites were unevenly distributed along Fu Creek and concentrated in the vicinity of waterfalls or rapid water streams where the noise was intense. The ambient noise levels at the frog calling sites were measured each night using an SPL meter (TES 1357; Tianjin Sheng Xing Tai Technology Co., Tianjin, China) and it ranged between 73.5 and 77.8 dB SPL RMS with A weighting.

2.2. Marking During the first few nights of the study, we captured 17 actively calling frogs, and marked their calling sites in the field (sites were marked with small, numbered plastic tags attached to trees or shrubs). Captured frogs were kept in individual holding tanks and placed indoors overnight. On the second day of capture, these frogs were tagged individually using two different colors of visible implantable elastomer

(Northwest Marine Technology, Shaw Island, WA, USA; Moosman and Moosman, 2006; Feng *et al.*, 2009b). The marker was implanted into the epidermal layer of the skin on their dorsal body surface, and/or fore- and hind-limbs. Marking was color and location coded for these frogs in order that individual frogs could later be identified in the field by shining UV light over the frog to visualize the individual-specific locations of fluorescent markers. Afterward, the frogs were released into the wild, to their individual calling sites.

Subsequently, each night we searched for marked males throughout our study site (L × W: 10 m × 3 m) using a UV light. The calling sites of individual males were stable and remained essentially unchanged throughout the study period. Thus, most evenings many of the labeled frogs could be spotted at the same boulders where they were initially captured. Individuals' calling rates fluctuated from one night to the next (0–80 calls), with some individuals maintaining a high calling rate across nights, e.g. active callers might emit up to 80 calls in an evening (~ 1 hour), whereas others were not so active in the chorus in some evenings. Frogs were positively identified before we recorded their vocalizations.

2.3. Sound recording Frogs' vocalizations were recorded using a digital audio recorder (Sound Devices 702, Sound Devices, WI, USA; frequency range: 10 Hz–96 kHz) and a miniature omni-directional condenser microphone (AKG model C417, AKG Acoustics, Vienna, Austria), using a sampling rate of 96 kHz and 16-bit accuracy. The microphone has a flat frequency response over 20–20 000 Hz and a drop of 10 dB at 30 000 Hz. The microphone was attached to a bamboo pole and placed at a distance of 30 cm from the calling male. Recordings were carried out mainly between 19 : 00 h and 22 : 30 h.

2.4. Call analysis In light of the presence of NLP, such as frequency jumps, subharmonics, deterministic chaos and biphonations, analysis of the vocal signals of *O. graminea* followed that of Feng *et al.* (2009b). First, for each call we used SELENA, a custom-designed software (Narins *et al.*, 2004; Feng *et al.*, 2006, 2009b), to display its narrow-band spectrogram and determine the call duration. Similar to *O. tormota* (Feng *et al.*, 2009b) and an earlier study in *O. graminea* (Shen *et al.*, 2011), visual inspection of the spectra of all recorded vocalizations showed that calls of *O. graminea* could be categorized into distinct call-types on the basis of the call duration, the range of the call fundamental frequency (f_0), the presence of NLP, and the number of notes in a call.

As one-note complex/steep-FM calls were the predominant call-type and contained NLP components, we chose these as the focus of further analysis. Other call types, e.g. one-note flat-FM and multi-notes, were used for analysis in vocal diversity. Using PRAAT (Boersma and Weenink, 2011), we first identified

the different temporal segments of a call (Figure 1) on the basis of their dynamic regimes. Segmentation was based on visual inspection of a call's narrowband spectrogram (Herzel, 1993) and associated Fourier frequency spectra, following earlier studies (Riede *et al.*, 2000, 2004; Titze *et al.*, 2008; Zollinger *et al.*, 2008; Feng *et al.*, 2009b). We noted that calls of *O. graminea* had the following dynamic regimes: harmonic phonation, deterministic chaos, frequency jump, biphonation, subharmonics, and signal break (Riede *et al.*, 1997; Wilden *et al.*, 1998; Fitch *et al.*, 2002; Feng *et al.*, 2009b). To segment a call, we simply placed segment borders at the boundaries between the different dynamic regimes (Figure 1). Following call segmentation, we determined the time and rate of occurrence of each phenomenon and measured the durations of the various segments (i.e., harmonic, subharmonic, deterministic chaos, frequency jump, biphonation). We additionally calculated these durations as percentages of the total call duration (i.e., their relative durations).

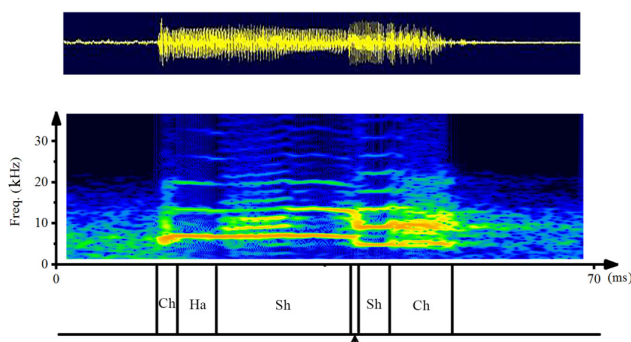


Figure 1 Oscillogram (top trace) and spectrogram (bottom trace) of a one-note steep-FM call showing how the call is segmented temporally into deterministic chaos (Ch), harmonic (Ha), subharmonic (Sh), and frequency jump (Fj) segments. At Fj it shows an abrupt change in the call f_0 ; at Sh it shows the presence of energy at $0.33 f_0$ (the first Sh segment) and $0.5 f_0$ (the second Sh segment); at Ch it displays noise-like spectra. This call does not show biphonation. The call was analyzed using Selenia, a sound analysis software (See Methods).

For each harmonic segment, we tracked the f_0 using the “pitch tracking” mode in PRAAT with 1-ms intervals. No f_0 measurement was made for NLP segments. During f_0 tracking, only the f_0 values that were perfectly aligned with the call's narrowband spectrogram were taken. If the f_0 track deviated from the first harmonic, the f_0 track was recomputed using different thresholds and octave cost values in PRAAT, until the deviation was completely eliminated. With tracking of f_0 , we measured the mean, maximum and minimum f_0 , as well as the difference and ratio between the maximum and minimum f_0 . Together, we measured a total of 14 temporal and spectral parameters for each call.

2.5. Statistical analysis We first employed Shapiro–Wilk

test to determine whether the data for each sound parameter were normally distributed (i.e., $P > 0.05$). It turned out that the data for many of the 14 sound parameters analyzed were not normally distributed, however, all data had the same shape distributions. Therefore, to determine whether or not vocalizations of individual males are distinct and contain individual vocal signatures, we carried out two different analyses. We first performed Kruskal–Wallis Analysis of Rank test, to determine whether vocal signals from different individuals were significantly different. For this, we included the data from 8 frogs from which we recorded > 40 calls each. To exclude the potential effect of ambient temperature on properties of vocalizations (Gerhardt 1978), data from frog #4 with an n of 16 were excluded as its calls were recorded from wide ranges of temperature (between 15 and 20.5 °C). To quantify call variability, within- and between-individual coefficient of variance of all acoustic parameter, i.e., CV_w and CV_b , were calculated. Within-individual variability can be attributed to many factors, including the ambient temperature (Gerhardt 1978) and background noise level. As recordings from 8 frogs were performed within narrow ranges of ambient temperature (16–19 °C) and noise level (74–77 dB SPL), much of the variability represented the differences in frog's neuromuscular control of the vocal apparatus at different times.

Subsequently, we carried out a stepwise discriminant function analysis (DFA) to predict group membership for each call. The result was a percentage documenting the correct assignment to individuals. For this, we removed several parameters that were correlated, e.g., absolute and relative durations of subharmonics. Out of the 14 sound parameters initially analyzed, we were thus left with 8 uncorrelated parameters (i.e., total duration, duration of the harmonic segments, duration of the subharmonic segments, duration of frequency jump segments, duration of the biphonic segments, average f_0 , f_0 Max, and f_0 Max– f_0 Min). As only 40 one-note steep-FM calls had been recorded from frog #16, to keep the sample size of the DFA more uniform (Feng *et al.*, 2009b), we took the first 40 calls from each of the eight males, thus a total of 320 calls. As a requirement of DFA was that the data be normally distributed, we first employed Shapiro–Wilk tests to examine whether the specific data sets ($n = 40$) for each sound parameter from eight individuals were normally distributed, and if not whether the non-normality was attributed to a skewness in the distribution or to the presence of outliers. We found that about half of the data sets were normally distributed; the remainders were not. However, all but $< 5\%$ of non-normality was due to skewness in the distribution. A recent simulation study (Pohar *et al.*, 2004) has shown that results of linear discriminant analysis (for data showing normal distribution) closely match results of logistic regression (for data

not showing normal distribution) if the non-normality is due largely to skewness of the distribution. As such, we chose to use DFA to determine group membership such that results of our analysis could be directly compared to those from earlier studies in other anuran species.

The a priori probability of correct assignment by the DFA was based on the total number of groups. Thus, the probability of a call belonging to 1 of 8 males was 12.5%. However, this procedure did not take into account the characteristics of the specific data (Titus *et al.*, 1984). Therefore, we calculated an alternative chance level on the basis of a randomized version of the actual data set (Solow, 1990). See <http://www.random.org> for a random sequence of the numbers from 1 to 320, using “sequence generator” function provided – this sequence was linked to the original data set. The number of calls per male was unchanged. The randomized set was then subjected to DFA, resulting in an average correct assignment (see Feng *et al.*, 2009b for details).

For DFA, we first loaded the data from all uncorrelated sound parameters (Riede, 1997; Feng *et al.*, 2009b). Next, to gain insight into the parameters which contributed the most to individual signatures, we performed analyses comparing the relative contributions of frequency-domain versus time-domain parameters, and the relative contributions of linear parameters versus NLP.

3. Results

We recorded a total of 1770 vocal calls from nine male *O. graminea*. Their vocalizations were diverse, consisting of two major classes: one-note calls and multi-note calls. One-note calls could be further divided into four sub-types on the basis of their spectral and temporal characteristics: complex/steep-FM calls that display complex and steep FM with 3–5 higher harmonics (Figure 2a) which shall be referred to simply as steep-FM calls from here on; flat-FM calls with 5–7 higher harmonics (Figure 2(b)); ultra-short (≤ 20 ms) low frequency flat-FM calls with 2–3 harmonics (Figure 2f); low-frequency (< 5 kHz) broadband calls (Figure 2g). Multi-note calls could similarly be divided into several subtypes on the basis of the spectrotemporal characteristics and number of notes (Figures 2c–e, h–j).

The majority of calls (68.0%) were one-note calls – these were emitted at various periods during the nightly chorus. In contrast, multi-note calls were heard only after chorus had built up later in the evening. The duration of one-note calls ranged widely, from 20 to 190 ms with a mean of (58.1 ± 35.2) ms. Distribution of call duration was skewed toward the lower end of the range (Figure 3a). The remainders of recorded vocal signals (32.0%) were multi-note calls. The inter note interval (or signal break duration) for these calls had a normal distribution

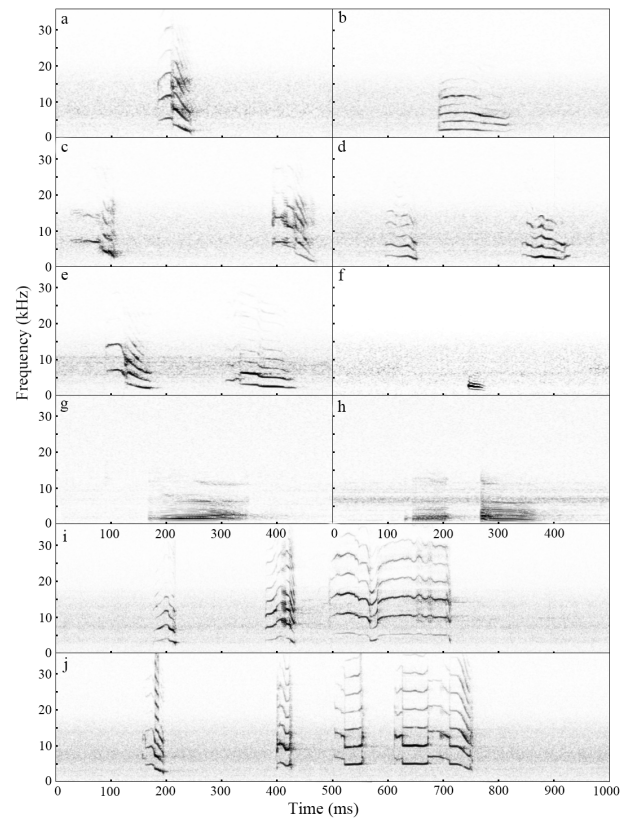


Figure 2 Spectrograms of vocalizations of *O. graminea* (Fuxi, China, 26 April–28 May 2015) show that their calls are diverse, comprising four subtypes of one-note calls (a: one-note complex/steep-FM call; b: one-note flat-FM call; f: ultra-short low-frequency flat-FM calls; g: low-frequency broadband calls) and six subtypes of multi-note calls (c: two-note complex/steep-FM call; d: two-note flat-FM call; e: two-note combination call; h: two-note low-frequency broadband call; i: three-note call; j: four-note call).

and ranged from 10 to 290 ms, with a mean of (142.4 ± 58.2) ms (Figure 3b). Breaks longer than 500 ms were taken as gaps (or intervals) between separate calls. Inter-call intervals ranged from a few second to tens of second, and to several minutes at nights when chorus was sparse.

One-note steep-FM (37.7%) and one-note flat-FM calls (30.0%) showed marked differences in their basic temporal and spectral characteristics (Table 1, Figure 4). Compared to flat-FM calls, steep-FM calls showed pronounced and diverse frequency modulation patterns and had shorter call duration (Figure 4f), higher f_0 (Figure 4e), and higher f_0 Max (Figure 4a), f_0 Min (Figure 4b), f_0 Max / f_0 Min (Figure 4d), as well as higher f_0 Max– f_0 Min (Figure 4c) [Mann-Whitney *U* tests, $P < 0.0001$]. The higher f_0 Max / f_0 Min and f_0 Max– f_0 Min basically reflected differences in the depths of frequency modulation of these two call-types. Additionally, steep-FM and flat-FM calls differed with respect to the degree to which NLP was contained in the call. Most steep-FM calls (Figures 2a, c)) exhibited

Table 1 Quantitative description (mean \pm SD) of fundamental temporal and spectral parameters of one-note steep-FM and one-note flat-FM calls of male *Odorrana graminea* (Fuxi, China, 26 April–28 May 2015). Mann-Whitney *U* tests were used to test the differences in the means between the two sub-types of one-note calls.

Sound Parameter	Steep-FM	Flat-FM	<i>P</i>
Total duration (ms)	48.8 \pm 19.6	69.9 \pm 45.4	< 0.0001
Average f_0 (kHz)	4.6 \pm 0.9	2.9 \pm 0.4	< 0.0001
f_0 Max (kHz)	7.1 \pm 1.2	3.6 \pm 0.5	< 0.0001
f_0 Min (kHz)	2.5 \pm 0.5	2.2 \pm 0.4	< 0.0001
f_0 Max – f_0 Min (kHz)	4.6 \pm 1.2	1.3 \pm 0.6	< 0.0001
f_0 Max / f_0 Min	3.0 \pm 0.7	1.7 \pm 0.4	< 0.0001

overt NLP. These calls showed one or more frequency jumps with other segments of the call featuring subharmonics and biphonations (Figure 1). In contrast, flat-FM calls (Figures 2b, 2d) were pure-tone like, showing only flat FM, and contained no NLP. The volume of data for one-note steep-FM calls was the largest as males emitted such calls throughout the evening. In light of the dominant presence of one-note steep-FM calls we selected them as the subject of further studies.

We recorded a total of 668 one-note steep-FM calls from 9 males. Calls of individual males were recorded across many evenings. The majority of these calls ($n = 577$; 86.4%) contained >1 NLP (Table 2). Only a small number of calls ($n = 91$; 13.6%) consisted of harmonic segments purely, without any NLP. This phenomenon was applicable to all frogs, except frog #17 for which more than half of his calls did not contain NLP.

A prevalent NLP component in steep-FM calls was frequency jumps. Most steep-FM calls that contained NLP (476 out of 577; 82.5%) exhibited one or more frequency jumps. At the opposite end, deterministic chaos was the least observed NLP component. Only two of 577 calls (0.3%) from two males

displayed deterministic chaos; for seven males, none of their calls contained deterministic chaos. Subharmonics and biphonations were intermediate in terms of their prevalence.

Table 2 shows that there were inter-individual differences in the numbers of occurrence of various NLP, especially those of subharmonics (0.9%–95%) and biphonation (2.5%–84.6%). The differences were also evidenced in terms of the durations of various NLP and of harmonic segments (Table 2). For all frogs, more than two-thirds (> 67%) of the duration of the call comprised harmonic segments, with the remaining consisted of NLP segments. The relative durations of subharmonics and biphonation showed particularly large inter-individual differences. In contrast, the relative duration of frequency jump was not as variable among individual males, and the relative duration for deterministic chaos was very low for all frogs.

Other than individual differences in the numbers of occurrence of NLP, steep-FM calls also displayed intra- and inter-individual differences in the frequency modulation patterns (Figure 5). A common feature of steep-FM calls was their general downward FM trend, but the FM pattern and the range of frequency excursion (or depth of FM), were highly variable. Calls of frog #17 featured first upward and later downward FM, and they showed no NLP other than frequency jumps (Figure 5d). Calls of other males tended to show a constant frequency in the beginning of the call followed by downward FM after the midpoint of the call (Figures 5b, d). Most calls displayed a downward frequency jump in the middle of the call, and the time of its occurrence was individual specific. Calls of some individuals showed additional downward or upward frequency jumps at other time points (Figures 5a, d). Whereas calls of some males showed a short biphonation segment at the beginning of the call (Figure 5(a)), calls of other males contained a biphonation segment near the end of the call

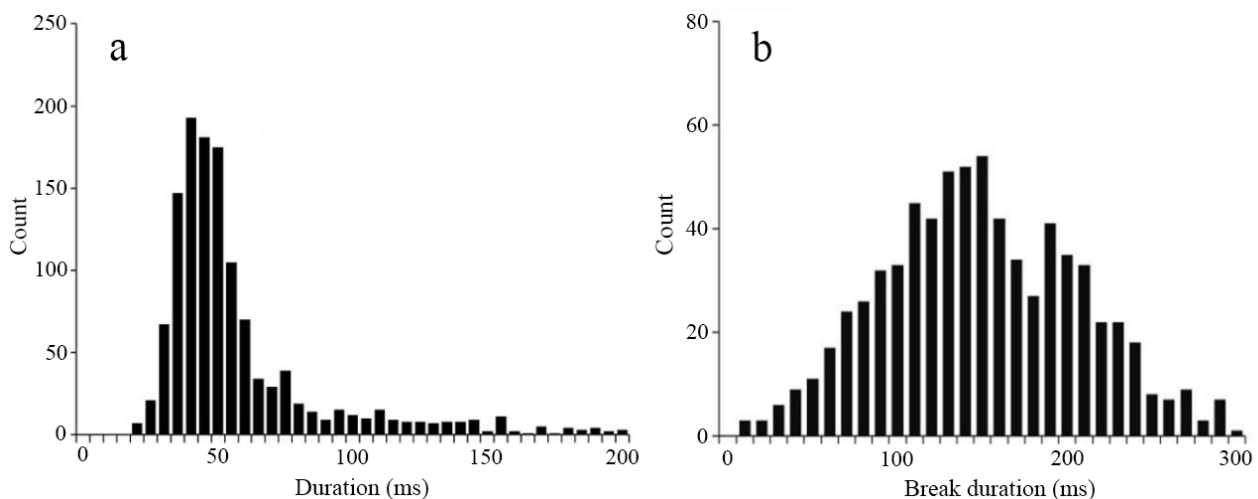


Figure 3 (a) Distribution of the duration of one-note calls, including one-note steep-FM calls, one-note flat-FM calls, ultra-short low-frequency broadband calls, and low-frequency broadband call. (b) Distribution of the inter-note interval (i.e., signal break) of multi-note calls.

(Figure 5b).

Quantitative measures of spectral parameters for individual males are shown in Table 3. The average f_0 of one-note steep-FM calls was 4.6 ± 0.9 kHz (range: 3.9–5.3 kHz). The f_0 , along with other spectral parameters, appeared to be individual specific as there existed large inter-individual differences.

To investigate whether calls of *O. graminea* contained individual signatures, we carried out Kruskal-Wallis Analysis of Rank test of 320 calls. The K-W test revealed significant differences between calls from individual frogs (Table 4). Results of K-W test further showed that all temporal and spectral parameters were significantly different between individuals.

To determine statistically whether the inter-individual variability was greater than intra-individual variability as observed qualitatively, we measured the between- (CV_b) and within-individual coefficients of variations (CV_w). The result showed that, for all acoustic parameters, the ratio CV_b/CV_w

was above 1, indicating that the CV_b was greater than the CV_w (Table 5).

To determine group membership for calls we performed a stepwise forward discriminant function analysis (DFA). Results of DFA showed that the average correct assignment of the original data set was 54.7%, indicating that on average 54.7% of the calls could be correctly assigned to the eight individuals (Table 6). This was higher than the chance-level correct assignment of 11.3%–13.1%. One-sided paired t-test showed that these two probabilities of correct assignment differed significantly ($P < 0.05$).

To gain insight into the parameters which contributed the most to individual signatures, we compare the relative contributions of frequency-domain versus time-domain parameters, we loaded the two sets of parameters separately into the DFA. We found that the mean correct assignment on the basis of temporal parameters alone was higher (49.4%) than

Table 2 Frequency of occurrence of the various NLP (shown on the left-half of the table) and the call duration and the relative durations (mean \pm SD) of the harmonic and various NLP segments (shown on the right-half of the table) in one-note steep-FM calls of *Odorrana graminea* (Fuxi, China, 26 April–28 May 2015). Abbreviations: SVL: snout-vent length; n: number of calls; Duration: total duration of calls; Ha: hamornic segment; Fj: frequency jump segment; Sh: subhamornic segment; Bp: biphonation segment; Ch: chaotic segment.

Frog ID	SVL (cm)	N	Ha	Ha (%)	Fj	Fj (%)	Sh	Sh (%)	Bp	Bp (%)	Ch	Ch (%)	Duration (ms)	Ha dur (%)	Sh dur (%)	Fj dur (%)	Bp dur (%)	Ch dur (%)
#2	5.62	65	1	1.5	53	81.5	31	47.7	55	84.6	1	1.5	42.2 \pm 8.5	66.8 \pm 16.7	10.5 \pm 13.9	3.4 \pm 2.2	19.0 \pm 11.4	0.3 \pm 2.2
#4	5.27	16	1	6.3	13	81.3	3	18.8	6	37.5	0	0	42.3 \pm 9.7	83.2 \pm 16.8	4.9 \pm 10.9	4.2 \pm 2.8	7.7 \pm 11.0	0 \pm 0
#7	5.44	95	1	1.1	60	63.2	17	17.9	60	63.2	1	1.1	58.9 \pm 38.7	81.3 \pm 14.0	2.3 \pm 5.2	2.5 \pm 2.9	13.9 \pm 15.0	0.1 \pm 0.5
#9	5.49	93	10	10.8	66	71	14	15.1	44	47.3	0	0	43.7 \pm 13.4	81.7 \pm 15.7	2.5 \pm 6.0	4.3 \pm 3.6	11.6 \pm 14.2	0 \pm 0
#11	5.08	56	10	17.9	41	73.2	11	19.6	10	17.9	0	0	55.8 \pm 15.1	92.1 \pm 9.5	3.3 \pm 8.2	2.5 \pm 2.3	2.1 \pm 4.8	0 \pm 0
#13	5.17	96	1	1	77	80.2	17	17.7	48	50	0	0	52.4 \pm 13.2	85.1 \pm 11.5	2.4 \pm 5.3	3.5 \pm 2.8	9.0 \pm 11.1	0 \pm 0
#15	5.02	101	7	6.9	90	89.1	2	2	33	32.7	0	0	44.4 \pm 14.4	85.9 \pm 10.7	0.4 \pm 3.1	6.9 \pm 3.5	6.7 \pm 9.6	0 \pm 0
#16	5.11	40	1	2.5	38	95	38	95	1	2.5	0	0	43.9 \pm 9.4	95.7 \pm 3.5	0.7 \pm 3.2	3.3 \pm 1.5	0.3 \pm 2.0	0 \pm 0
#17	5.45	106	59	55.7	38	35.8	1	0.9	16	15.1	0	0	48.3 \pm 11.9	95.9 \pm 7.2	0.1 \pm 1.4	1.3 \pm 1.9	2.7 \pm 6.6	0 \pm 0

Table 3 This table shows a quantitative description (mean \pm SD) of the spectral characteristics of one-note steep-FM calls of male *Odorrana graminea* (Fuxi, China, 26 April–28 May 2015). n: the number of calls recorded and analyzed; f_0 : call fundamental frequency.

Frog ID	n	Mean f_0 (kHz)	f_0 Max (kHz)	f_0 Min (kHz)	f_0 Max – f_0 Min (kHz)	f_0 Max / f_0 Min
#2	65	4.8 \pm 1.0	7.5 \pm 0.9	2.8 \pm 0.8	4.7 \pm 1.1	2.8 \pm 0.6
#4	16	5.3 \pm 1.0	7.2 \pm 0.7	3.1 \pm 0.2	4.1 \pm 1.3	2.5 \pm 0.5
#7	95	5.1 \pm 0.9	7.6 \pm 0.7	2.5 \pm 0.3	5.1 \pm 0.8	3.1 \pm 0.5
#9	93	4.3 \pm 0.7	7.3 \pm 0.2	2.4 \pm 0.5	5.0 \pm 1.4	3.3 \pm 0.9
#11	56	4.9 \pm 1.1	7.5 \pm 1.1	2.4 \pm 0.3	5.1 \pm 1.0	3.2 \pm 0.5
#13	96	5.0 \pm 1.1	7.9 \pm 1.0	2.4 \pm 0.4	5.5 \pm 1.0	3.4 \pm 0.7
#15	101	4.0 \pm 0.5	5.7 \pm 0.5	2.5 \pm 0.3	3.2 \pm 0.5	2.3 \pm 0.3
#16	40	4.5 \pm 0.7	7.4 \pm 0.6	2.1 \pm 0.3	5.4 \pm 0.6	3.7 \pm 0.5
#17	106	4.4 \pm 0.6	6.4 \pm 0.9	2.3 \pm 0.4	4.0 \pm 0.9	2.8 \pm 0.6

that based on spectral parameters alone (36.9%). Both were above chance level. We also calculated and compared the mean correct assignments for linear versus nonlinear parameters. We found that the nonlinear parameter set yielded a higher mean correct assignment (44.1%) compared to that for linear parameter set (31.6%).

Figure 6 shows a plot of male calls in a three-dimensional signal space defined by the canonical scores. The figure shows that there was overlap among individuals, but the separation in space was sufficiently large to allow above-chance assignment of the DFA.

4. Discussion

Results of the present study show that calls of *O. graminea* are comprise two major classes (Figure 2). Similar to the vocal signals of male (Feng *et al.*, 2009b) and female *O. tomota* (Zhang *et al.*, 2017), spectrotemporal features of their calls are highly variable intra- and inter-individual variability, such as one-note steep-FM calls, three-note and four-note calls as well as a couple two-note calls. In addition, a notable feature of the vocalization of male *O. graminea* is that, compared to the advertisement calls of *O. tormota* (Feng *et al.*, 2009b; Zhang *et al.*, 2017) and

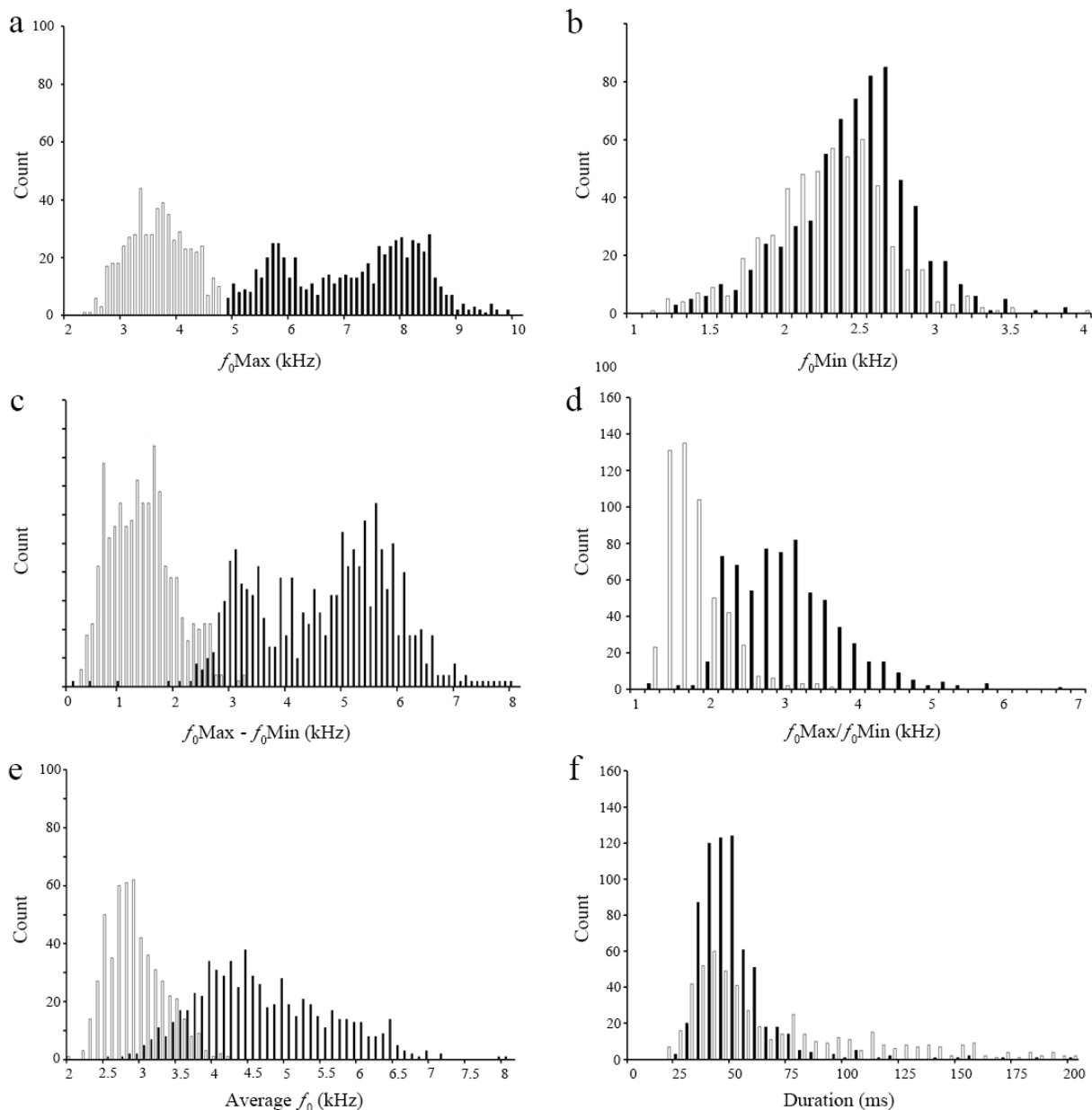


Figure 4 Distributions of various basic spectral and temporal parameters of one-note steep-FM calls (in black) and one-note flat-FM calls (in grey). (a) distribution of $f_0\text{Max}$; (b) distribution of $f_0\text{Min}$; (c) distribution of $f_0\text{Max}-f_0\text{Min}$; (d) distribution of $f_0\text{Max}/f_0\text{Min}$; (e) distribution of average f_0 ; (f) distribution of the call duration.

Table 4 Results of Kruskal-Wallis Analysis of Rank test examining between-individual variability ($n = 320$; $df = 7$).

Sound Parameters	X^2	p
*Total duration (ms)	48.637	< 0.0001
Harmonic segment duration (ms)	85.038	< 0.0001
*Harmonic segment duration (%)	134.329	< 0.0001
*Subharmonic segment duration (ms)	46.335	< 0.0001
Subharmonic segment duration (%)	47.373	< 0.0001
Frequency jump segment duration (ms)	90.624	< 0.0001
*Frequency jump segment duration (%)	97.761	< 0.0001
*Biphonic segments duration (ms)	90.624	< 0.0001
Biphonic segments duration (%)	95.577	< 0.0001
*Average f_0 (Hz)	44.761	< 0.0001
* f_0 Max (Hz)	100.327	< 0.0001
f_0 Min (Hz)	69.665	< 0.0001
* f_0 Max - f_0 Min (Hz)	114.482	< 0.0001
f_0 Max / f_0 Min	118.23	< 0.0001

Note: * indicates the sound parameters used in the discriminant function analysis.

Table 5 Coefficients of variations of call duration and durations of various harmonic and NLP segments (absolute measure only) of one-note steep-FM calls of *Odorrana graminea*. Abbreviations: Ha: hamornic segment; Fj: frequency jump segment; Sh: subhamornic segment; Bp: biphonation segment.

Parameter	Mean CV _w	Mean CV _b	CV _b /CV _w
Total duration (ms)	34.38 ± 14.57 (21.28–64.26)	41.29	1.20
Duration of Ha (ms)	40.07 ± 17.36 (20.84–75.72)	48.25	1.20
Duration of Sh (ms)	43.47 ± 15.73 (26.12–60.89)	55.34	1.27
Duration of Fj (ms)	43.77 ± 13.33 (20.41–64.11)	54.11	1.24
Duration of Bp (ms)	34.29 ± 14.28 (14.45–55.46)	47.04	1.37
Average f_0 (Hz)	18.49 ± 3.10 (14.41–22.28)	20.66	1.12
f_0 Max (Hz)	13.55 ± 3.38 (8.74–19.37)	16.83	1.24
f_0 Min (Hz)	15.83 ± 3.67 (10.28–20.81)	17.91	1.13
f_0 Max- f_0 Min (Hz)	20.21 ± 6.62 (10.58–30.42)	26.29	1.30

other anuran species studied previously (Bee *et al.*, 1999; Bee and Gerhardt, 2001; Gasser *et al.*, 2009; Bee *et al.*, 2016), the duration of one-note steep-FM calls consisted of NLP components is very short (average of 48.8 ms). As a result, it limits the numbers of occurrence of the various NLP, as well as the variety of NLP

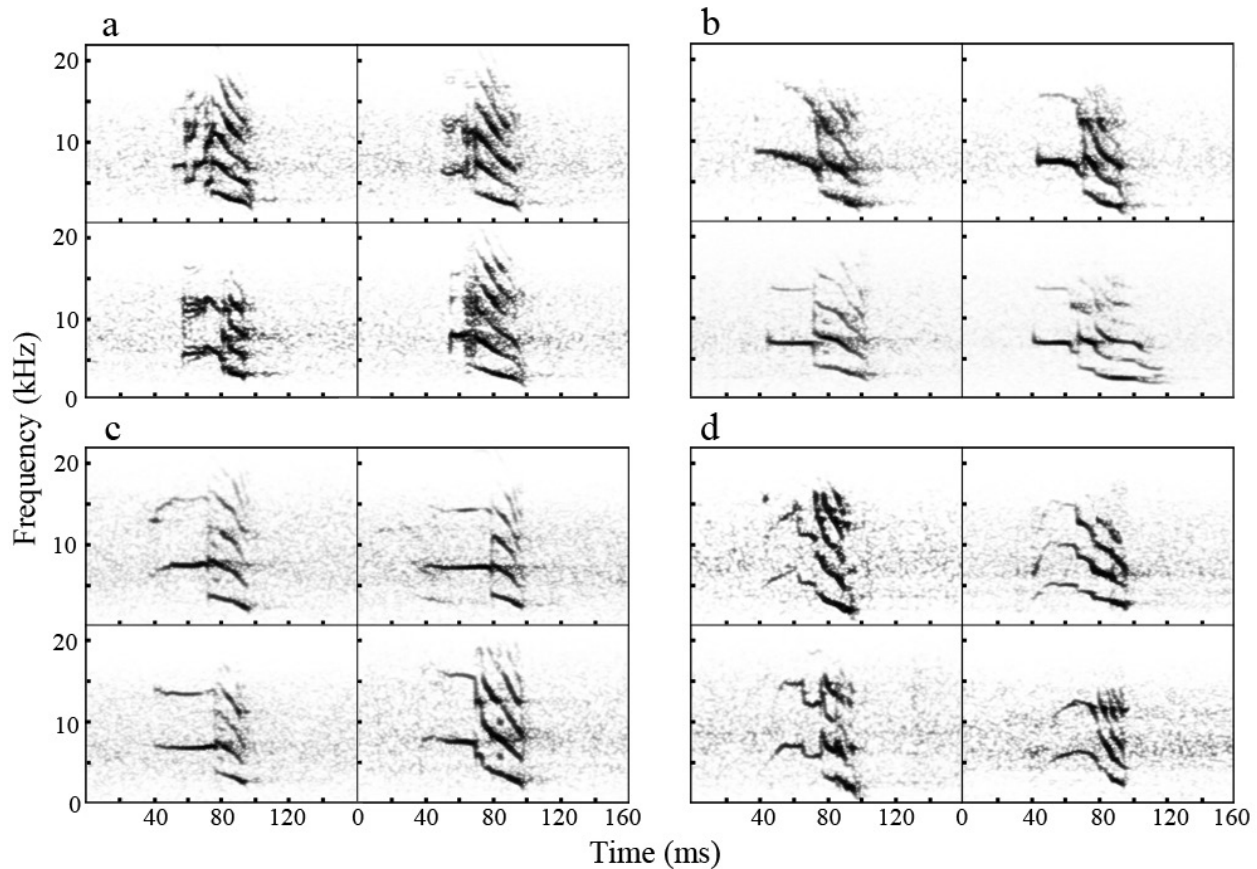


Figure 5 Distributions of various basic spectral and temporal parameters of one-note steep-FM calls (in black) and one-note flat-FM calls (in grey). (a) distribution of f_0 Max; (b) distribution of f_0 Min; (c) distribution of f_0 Max- f_0 Min; (d) distribution of f_0 Max/ f_0 Min; (e) distribution of average f_0 ; (f) distribution of the call duration.

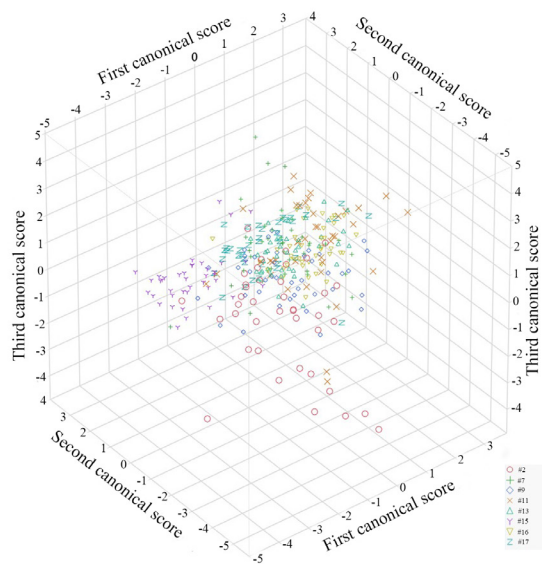


Figure 6 Vocalizations of male *O. graminea* (Fuxi, China, 26 April–28 May 2015) in a three-dimensional signal space defined by canonical scores.

that can show up in the vocal signal. In fact, the numbers of occurrence of chaos and subharmonics are very low when compared to male *O. tormota* (Feng *et al.*, 2009b), another anuran species that exhibit pronounced NLP in their advertisement calls.

The proportion of calls containing at least one NLP in male *O. graminea* (86.4%) is comparable to that for male *O. tormota*, for whom 93% of vocal signals exhibit NLP (Feng *et al.*, 2009b). For anurans, nonlinearities originated either from the vocal folds themselves, or from an interactive coupling between the folds and the surrounding airspaces, and the allometric relationships between body size and vocal apparatus could affect the

occurrence and strength of nonlinearities in acoustic signals (Cazau *et al.*, 2016). For example, in Darwin's frogs (*Rhinoderma darwini*), the smaller individuals had higher proportions of the relative duration of chaos, suggesting the body size has an influence on NLP components (Serrano *et al.*, 2019). The factors contributing to the occurrence of NLP in male *O. graminea* remain to be determined empirically. However, the anatomical variation of vocal folds between *O. graminea* and *O. tormota* might contribute to the differences on proportions of NLP among them.

Our study showed that vocalization of male *O. graminea* contained NLP and exhibited individual signatures. The first result differs from that of Shen *et al.* (2011). Their study did not uncover NLP in any of the calls recorded from male *O. graminea*. A contributing factor to this discrepancy is the fewer number of call-types found in their study. We have observed that only select call-types, in particular one-note steep-FM calls, contain NLP, and many other call-types do not. The variability across individuals is another contributing factor, as calls of some male exhibit few or no NLP whereas other males emit calls many of which exhibiting prominent NLP.

It has been suggested that the presence of NLP can contribute to individual signatures (Wilden *et al.*, 1998; Fitch *et al.*, 2002; Volodina *et al.*, 2006). Results of the present study in male *O. graminea*, as well as results from male (Feng *et al.*, 2009b) and female *O. tormota* (Zhang *et al.*, 2017), provide support for this principle, as the vocalizations of these frogs all carry individual signatures, and for these species the relative abundance of NLP components is individual specific. Further, results of comparative DFA in the present study directly show that NLP contributes more to individual signatures than linear sound parameters such as the average and maximum f_0 , $f_0\text{Max}$ — $f_0\text{Min}$, and the call duration.

Table 6 This table shows the results of discriminant function analysis. The results were based on correct classification of 320 calls from eight frogs based on eight sound parameters. Shown is the correct classification for the original data set as well as of the randomized data set (#1–#5). The one-sided *t*-test compares the correct classifications of the original data set with the respective random data sets. Significance level $P < 0.05$.

Frog ID	<i>n</i>	Original date set	Random date set 1	Random date set 2	Random date set 3	Random date set 4	Random date set 5
2#	40	65	20	7.5	10	10	15
7#	40	47.5	10	10	15	7.5	15
9#	40	22.5	15	15	10	12.5	15
11#	40	47.5	15	5	10	12.5	7.5
13#	40	32.5	20	17.5	12.5	17.5	12.5
15#	40	82.5	7.5	15	10	15	17.5
16#	40	62.5	5	7.5	12.5	10	5
17#	40	77.5	10	12.5	10	17.5	17.5
Mean correct assignment (%)		54.7	12.8	11.3	11.3	12.8	13.1
One-sided paired <i>t</i> -test			$t = 4.86$	$t = 5.49$	$t = 5.69$	$t = 5.67$	$t = 5.75$
			$P = 0.001$	$P = 0.001$	$P = 0.001$	$P = 0.001$	$P = 0.001$

DFA of calls of male *O. graminea* have shown that their calls are statistically discriminable, as has been shown for various anuran species (Bee *et al.*, 2016). Stepwise DFA can correctly classify ~55% of the calls to eight individuals. This classification level is comparable to that observed in male concave-eared torrent frogs (55% for 6 frogs; Feng *et al.*, 2009a), male green frogs (52%–100%; Bee *et al.*, 1999) and male aromobatid frog (65%–94%; Gasser *et al.*, 2009), but is lower than that of female concave-eared torrent frogs (87% for 5 frogs; Zhang *et al.*, 2017) and male bullfrogs (92% for 5 males; Bee and Gerhardt, 2001). The classification success of *O. graminea* falls in the lower range comparatively. This is not surprising due to the larger number of individuals used in the DFA compared to some previous studies. Comparison of DFA data in various anuran species reveals that there is a trend of decreasing DFA value with an increase in the number of individuals sampled. This trend is compatible with the conclusion reached by a systematic study in little owls in which Linhart and Šálek (2017) demonstrated empirically that DFA value is smaller when the number of individuals is increased. In addition, because NLP contributes more to individual signatures than that of linear sound parameters, the shorter duration of one-note steep-FM calls containing NLP components can be expected to reduce the individual distinctiveness for *O. graminea*. Lastly, discrimination only based on one call type and its properties would probably be underestimated because other call types and their signal properties might have some potential to allow discrimination among male *O. graminea* (Bee and Gerhardt, 2001). Future studies should examine the importance of other acoustic features as potential recognition cues.

For male *O. tormota*, males seem to have ability to discriminate nearby neighbors from strangers acoustically (Feng *et al.*, 2009a) due in part to the presence of individual signatures in their calls (Feng *et al.*, 2009b). Whether or not male *O. graminea* possess this discrimination ability remains to be determined. Our field observations reveal that males *O. graminea* occupy the same calling sites night-in and night-out, and their calling sites remain unchanged throughout a single reproductive season. They tolerate the presence of neighbor vocalizing males. At the peak of chorusing activity, a male may be surrounded by 3–5 actively calling males within a 30 cm radius in 3D. Furthermore, we have also observed several cases of male-male physical combat. Thus, they appear to display “dear enemy phenomenon” (Fisher, 1954). Validation of this hypothesis, however, requires experimental evidence, for instance through playing back multiple calls from “stranger” versus calls from a familiar neighbor in the field from the neighbor’s original calling site (Bee *et al.*, 2016).

We found that whereas one-note steep-FM calls possess harmonics in the ultrasonic range and many exhibit NLP,

one-note flat-FM calls uniformly do not have energy in the ultrasonic range nor contain NLP (Figure 2). This finding raises an interesting question. Namely, whether the extension of call energy into the ultrasonic range relate to the occurrence of NLP, as the appearance of NLP in anurans has so far been observed mostly to species which produce ultrasound. In other words, are elements of the vocal fold responsible for the production of ultrasound shared with those elements responsible for the production of NLP, or are these two groups independent? A survey of comparative literature in anurans indicates that this relationship appears tenuous. Whereas most vocalizations of male (Narins *et al.*, 2004; Feng *et al.*, 2009b) and female *O. tormota* (Zhang *et al.*, 2017) as well as those of male *O. graminea* (this study) have harmonics in the ultrasonic range and they also exhibit prominent NLP, those of *Huia cavitympanum* (Arch *et al.*, 2008) show no NLP despite having energy well into the ultrasonic range. As large inter- and intra-individual variability can obscure the observation of NLP, as evidenced for female *O. tormota* (Shen *et al.*, 2008) and male *O. graminea* (Shen *et al.*, 2011), whether the vocalizations of *Huia cavitympanum* are truly devoid of NLP should be reexamined before reaching a safe conclusion regarding the relationship between ultrasonic calls and occurrence of NLP. In general, further research is needed to illuminate the function, the behavioral significance, and the mechanisms of production of NLP in various anuran species.

Beyond contributing to individual signatures, the function of NLP remains poorly understood. It has been suggested that NLP boost the auditory salience and provide cues to the fitness of vocalizers (Wilden *et al.*, 1998; Riede *et al.*, 2000). Exactly what are the primary and secondary function of NLP is well deserved of a further investigation.

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References

- Arch V. S., Grafe T. U., Narins P. M. 2008. Ultrasonic signalling by a Bornean frog. *Biol Lett*, 4: 19–22
- Bee M. A., Perrill S. A., Owen P. C. 1999. Size assessment in

- simulated territorial encounters between male green frogs (*Rana clamitans*). *Behav Ecol Sociobiol*, 45: 177–184
- Bee M. A., Gerhardt H. C. 2001. Neighbour-stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): i. acoustic basis. *Anim Behav*, 62: 1129–1140
- Bee M. A., Reichert M. S., Tumulty J. 2016. Assessment and recognition of rivals in anuran contests. *Adv Study Behav*, 48: 161–249
- Boersma P., Weenink D. PRAAT: doing phonetics by computer (Version 5.2.22) [Computer program]. Retrieved from <http://www.praat.org>
- Briefer E., Aubin T., Lehongre K., Rybak F. 2008. How to identify dear enemies: the group signature in the complex song of the skylark *Alauda arvensis*. *J Exp Biol*, 211: 317–326
- Chen B. H. 1991. *Anhui Amphibia and Reptilia*. Anhui Press of Sciences and Technology, China (In Chinese)
- Falls J. B. 1982. Individual recognition by sound in birds. In: *Acoustic Communication in Birds* (Kroodsmas, D. E. & Miller, E. H., eds). Academic Press, New York, NY, pp. 237–278
- Feng A. S., Narins P. M., Xu C. H., Lin W. Y., Yu Z. L., Qiu Q., Xu Z. M., Shen J. X. 2006. Ultrasonic communication in frogs. *Nature*, 440: 333–336
- Feng A. S., Arch V. S., Yu Z. L., Yu X. J., Xu Z. M., Shen J. X. 2009a. Neighbor–stranger discrimination in concave-eared torrent frogs, *Odorrana tormota*. *Ethology*, 115: 851–856.
- Feng A. S., Tobias R., Arch V. S., Yu Z. L., Xu Z. M., Yu X. J., Shen J. X. 2009b. Diversity of the vocal signals of concave-eared torrent frogs (*Odorrana tormota*): evidence for individual signatures. *Ethology*, 115: 1015–1028.
- Fisher J. 1954. Evolution and bird sociality. Pp. 71–83 in *Evolution as a Process* (J. Huxley, A.C. Hardy, and E.B. Ford, eds.). Allen & Unwin, England
- Fitch W. T., Neubauer J., Herzel H. 2002. Calls out of chaos: the adaptive significance of nonlinear phenomena in mammalian vocal production. *Anim Behav*, 63: 407–418
- Gasser H., Amézquita A., Hödl W. 2009. Who is calling? Intraspecific call variation in the aromobatid frog *Allobates femoralis*. *Ethology*, 115: 596–607
- Gerhardt H. C. 1978. Temperature coupling in the vocal communication system of the gray tree frog, *Hyla versicolor*. *Science*, 199: 992–994
- Herzel H. 1993. Bifurcations and chaos in voice signals. *Appl Mech Rev*, 46: 399–413.
- Linhardt P., Šálek M. 2017. The assessment of biases in the acoustic discrimination of individuals. *Plos One*, 12:e0177206
- Moosman D. L., Moosman Jr. P. R. 2006. Subcutaneous movements of visible implant elastomers in wood frogs (*Rana sylvatica*). *Herpetol Rev*, 37: 300–301
- Narins P. M., Feng A. S., Lin W., Schnitzler H., Denzinger A., Suthers R. A., Xu C. 2004. Old World frog and bird vocalizations contain prominent ultrasonic harmonics. *J Acoust Soc Am*, 115: 910–913.
- Pohar M., Blas M., Turk S. 2004. Comparison of logistic regression and linear discriminant analysis: A simulation study. *Metodoloski Zvezki* 1: 143–161
- Riede T. 1997. Individual differences in the vocalization of Japanese macaque infants (*Macaca fuscata*). *Primate Report*, 47: 31–47
- Riede T., Wilden I., Tembrock G. 1997. Subharmonics, biphonations, and frequency jumps common components of mammalian vocalization or indicators for disorders? *Zeitschrift fur Säugetierkunde* 62(Suppl. II): 198–203
- Riede T., Herzel H., Mehwald D., Seidner W., Trumler E., Böhme G. 2000. Nonlinear phenomena in the natural howling of a dog–wolf mix. *J Acoust Soc Am*, 108: 1435–1442
- Riede T., Owren M. J., Arcadi A. C. 2004. Nonlinear acoustics in pant hoots of common chimpanzees (*Pan troglodytes*): frequency jumps, subharmonics, biphonation, and deterministic chaos. *Am J Primatol*, 64: 277–291
- Shen J. X., Feng A. S., Xu Z. M., Yu Z. L., Arch V. S., Yu X. J. 2008. Ultrasonic frogs show hyperacute phonotaxis to female courtship calls. *Nature*, 453: 914–917
- Shen J., Yu Z. L., Feng A. S., Narins P. M. 2011. Large odorous frogs (*Odorrana graminea*) produce ultrasonic calls. *J Comp Physiol A*, 197: 1027–1030
- Solow A. R. 1990. A randomization test for misclassification probability in discriminant analysis. *Ecology*, 71: 2379–2382
- Temeles E. J. 1994. The role of neighbours in territorial systems: when are they ‘dear enemies’? *Anim Behav*, 47: 339–350
- Titus K., Mosher J. A., Williams B. K. 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. *Am Midl Nat*, 111: 1–7
- Titze I., Riede T., Popolo P. 2008. Nonlinear source-filter coupling in phonation: vocal exercises. *J Acoust Soc Am*, 123: 1902–1915
- Volodina E. V., Volodin I. A., Isaeva I. V., Unck C. 2006. Biphonation may function to enhance individual recognition in the dhole, *Cuon alpinus*. *Ethology*, 112: 815–825
- Wilden I., Herzel H., Peters G., Tembrock G. 1998. Subharmonics, biphonation, and deterministic chaos in mammal vocalization. *Bioacoustics*, 9: 171–196
- Zhang F., Chen P., Chen Z. Q., Zhao J. 2015. Ultrasonic frogs call at a higher pitch in noisier ambient. *Curr Zool*, 61: 996–1003
- Zhang F., Zhao J., Chen P., Chen Z. Q., Chen Y. Y., Feng A. S. 2016. Heterogeneity of vocal sac inflation patterns in *Odorrana tormota* plays a role in call diversity. *J Acoust Soc Am*, 139: 1018–1023
- Zhang F., Zhao J., Feng A. S. 2017. Vocalizations of female frogs contain nonlinear characteristics and individual signatures. *Plos One*, 12:e0174815
- Zhang F., Yuan C., Feng A. S. 2020. Female concave-eared torrent frogs prefer smaller males. *J Zool*, doi:10.1111/jzo.12775
- Zollinger S. A., Riede T., Suthers R. A. 2008. Two-voice complexity from a single side of the syrinx in northern mockingbird *Mimus polyglottos* vocalizations. *J Exp Biol*, 211: 1978–1991

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